# SPECIALIA

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## The occurrence of 12, 13-epoxytrichothecenes in seeds of safflower infected with Fusarium oxysporum f. sp. carthami

S. Ghosal[1], D. K. Chakrabarti and K. C. Basu Chaudhary

*Department of Pharmaceutics, Banaras Hindu University, Varanasi-5 (India), and Department of Plant Pathology, Banaras Hindu University, Varanasi-5 (India), 19 August 1976*

*Summary.* From the seeds of safflower (Carthamus tinctorius Linn.), infected with Fusarium oxysporum f. sp. carthami, 3 toxic compounds have been isolated in quantities sufficient to cause mycotoxicosis on prolonged ingestion. 2 of these have been identified as diacetoxyscirpenol and T-2 toxin, while the third one has also been partially characterized as a 12,13-epoxytrichothecene. Additionally, the incidence of secondary fusarial infection of healthy seeds due to contamination with the infected ones has been reported for the first time.

Fusarium oxysporum Schl. f. carthami Klisiewicz et Houston was reported[2,3] as a causal organism for the wilt disease of safflower (Carthamus tinctorius Linn.). The occurrence of 2 12,13-epoxytrichothecenes, viz., diacetoxyscirpenol and T-2 toxin, in the culture fluid of the fungus was reported recently[4]. Also, the toxic symptoms in safflower after the natural infection and those produced upon administration of the trichothecenes, isolated from the culture filtrates, were found[5] to be very similar. These observations prompted us to investigate whether the toxins are produced in the host tissues after the fungal infection.

We now report the isolation and characterization of 3 toxic substances from the seeds of safflower infected with F. oxysporum f. sp. carthami (IMI 166917). Additionally, the incidence of secondary infection of healthy seeds of safflower due to contamination with infected ones has been reported.

The seeds were harvested from different parts of Varanasi and Mirzapur districts of Uttar Pradesh (India) during March 1974. In a typical experiment, the isolation of the toxic substances from the infected seeds (100 g) was accomplished by water extraction of the seed-mycelial mixture in a high speed blender. The aqueous filtrate and the wet cake remaining after filtration were repeatedly blender extracted with chloroform. The combined chloroform extracts was processed in the usual way[4] to give a yellow oil (0.112 g) that was biologically active[6]. Preliminary purification of the oily substance was carried out by passing its ethyl acetate solution (10 ml) through a column of silica gel (BDH, 60–120 mesh, $1.8 \times 22$ cm) using ethyl acetate-hexane (1:1) as the eluent. Fractions (25 ml) were collected. The first 2 fractions were kept aside since they exhibited only weak biological activity. The concentrate from fractions 3–6, an oil, which was biologically active, showed 3 prominent spots around $R_f$ 0.4–0.7 on analytical TLC (silica gel G, E. Merck; $CHCl_3$-MeOH, 95:5). The TLC plates showed purple-violet colours of varying intensities when sprayed with p-dimethylamino benzaldehyde or p-anisaldehyde in ethanolic hydrochloric acid, and brown changing to lead-grey spots with concentrated sulphuric acid with subsequent heat treatment. The colours developed were typical of 12, 13-epoxytrichothecenes. A portion (50 mg) of the oil from chromatography (fractions 3–6) was dissolved in chloroform (2 ml) and subjected to preparative layer chromatography (PLC). 3 dull yellow bands (I–III) appeared within the above-mentioned $R_f$-zone. These were cut and eluted with chloroform. The whole experiment was repeated several times to collect small quantities of the 3 compounds for spectral analyses and the biological testing.

The band I ($R_f \sim 0.4$) afforded a gummy residue (major component, 7 mg/kg seeds) which showed TLC and spectral behaviour identical with an authentic sample of diacetoxyscirpenol[7]. The residue from band II ($R_f \sim 0.5$), a minor component, showed a dull violet colour with the p-anisaldehyde reagent and a brown changing to grey spot with sulphuric acid treatment. It exhibited only an end absorption in its u.v. spectrum. On rat skin dermal toxicity test[6], reddish weals appeared when a 200 mcg dose of this substance was applied on the shaved skin of albino rats. These properties indicated it to be a member of the 12, 13-epoxytrichothecenes. The residue from band III ($R_f \sim 0.7$), also an oil (3 mg/kg of seeds), showed UV, IR and mass spectra which were indistinguishable from the reference spectra[8] of T-2 toxin.

On the rat skin bioassay test[6] with the total oil from the infected seeds, doses of 0.1, 0.2, and 0.4 mg of the substance were applied to the shaved skin of albino rats (80–120 g). 2 rats were used at each dose level. Swollen reddish weals were observed on all six rats on the second

1 Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, (India). To whom correspondence should be directed.
2 I. M. Klisiewicz, Phytopathology *53*, 1046 (1963).
3 A. K. Singh, D. K. Chakrabarti and K. C. Basu Chaudhary, Curr. Sci. *44*, 397 (1975).
4 S. Ghosal, D. K. Chakrabarti and K. C. Basu Chaudhary, J. pharm. Sci. *65*, 160 (1976).
5 D. K. Chakrabarti, K. C. Basu Chaudhary and S. Ghosal, Experientia *32*, 608 (1976).
6 R.-D. Wei, E. B. Smalley and F. M. Strong, Appl. Microbiol. *23*, 1029 (1972).
7 Kindly provided by Dr D. Hauser and Dr A. Closse, Sandoz, Basel (Switzerland).
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day which became progressively severe in case of dose 0.4 mg developing into a heavy scab 3 days after the treatment. Scab had also formed on the other 4 rats. While the 0.4 mg dose left pinkish scars, the spots treated with the 2 lower doses developed small tufts of hair with time. On increasing the dose of the toxic oil (1 mg), the animals died within 5–6 days. The observed level of toxins (about 3–5 mcg/g of seeds) when consumed through the oral route may not be sufficient to cause symptoms of a mycotoxicosis immediately, but prolonged ingestion may be fatal[9] and is, therefore, cause for alarm.

Another important observation made during this study is the incidence of secondary infection of safflower seeds by contamination with the infected ones. Surface-sterilized (0.1% aq. $HgCl_2$) healthy seeds of safflower were kept on the fungal colony on a PDA medium. On the second day, the seeds were removed and divided into 2 parts. One part was kept with an equal number of healthy seeds in aseptic conditions, at 21 °C for 30 days. The spores and mycelia from the second part were removed by successive washing with mercuric chloride solution and sterile distilled water. These were then kept with an equal number of healthy seeds as described above. After incubation for 30 days, the seeds were transferred aseptically on sterilized PDA plates and the latter were incubated at 21 °C. Within 72 h, the fungus appeared on all seeds and formed colonies around them. The presence of the fungus inside and outside the seeds was also tested by the blotter technique[10]. These results would seem to indicate that the seeds which carry spores on their surface and those bearing mycelium on their parenchymatous tissues can both serve as sources of the inoculum. Consequently, the fungus is capable of infecting not only immature seeds in field conditions, it can also penetrate the hard core of mature seeds during harvest and storage. Fusarium, which was regarded as one among well-known 'field fungi'[11], has thus been found, for the first time, to invade seeds even during storage. In tropical countries, where warm and moist climates prevail, the incidence of secondary infection of seeds could be very high and, therefore, would involve high toxin risk in man and animals.

9 C. J. Mirocha and C. M. Christensen, A. Rev. Phytopath. *12*, 303 (1974).
10 P. Neergaard, Proc. int. Seed Test. Ass. *35*, 19 (1970).
11 C. M. Christensen and H. H. Kaufmann, A. Rev. Plant Path. *3*, 69 (1965).

## The absolute configuration of SU 23397: A novel neuroleptic agent

N. K. Chaudhuri[1,2], T. J. Ball[1] and N. Finch

*Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit (New Jersey 07901, USA),*
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*Summary.* The absolute configuration of a novel chiral neuroleptic agent SU 23397 (**I**) was determined by ORD comparison of (+)-5-methoxy dihydro coumarilic acid (**VIII**), a synthetic precursor of SU 23397 (**I**), with (+)-dihydro coumarilic acid, whose absolute configuration is known[9]. This assignment was confirmed by oxidative degradation of (+)-5-methoxy dihydro coumarilic acid **VIII** to D-(+)-malic acid.

The antipsychotic activity of a novel chiral neuroleptic agent, SU 23397 (**I**) was recently described by 2 clinical groups[3,4]. It has been previously reported with other potent neuroleptic agents, e.g. octoclothepin[5] and butaclamol[6], that only one enantiomer of the racemic drug is active. SU 23397 (**I**) is the neuroleptically active (–)-enantiomer of such a racemic drug (**II**). We now describe the determination of the absolute configuration of SU 23397 (**I**) by both a chiro optical method and by degradation to a fragment of known absolute configuration.

SU 23397 (**I**) was originally prepared by Huebner by resolution via the N-acetyl-l-phenyl alanine salt of the racemate **II**[7]. The racemate **II** was prepared by alkylation of the commercially available (Aldrich Chemical Co.) triazaspirodecanone (**III**) with the bromo dihydro benzofuran **IV**[7] (scheme 1). The bromodihydro benzofuran **IV** was prepared by reaction of 2-allyl-4-methoxy phenol, obtained from a Claisen rearrangement, with bromine in the presence of a base. This compound **IV** is also available commercially (Alfred Bader Chemicals).

In order to provide a more efficient synthesis of SU 23397 (**I**), it was necessary to introduce the chirality earlier in the synthetic scheme and via an intermediate which could be epimerized and thus be recycled. (±)-5-methoxy dihydro coumarilic acid **VII** was such an intermediate. Dihydro coumarilic acid had been resolved via its amphetamine salts[8] and the absolute configuration of the enantiomers determined[9]. (±)-5-Methoxy coumarilic acid (**VI**), m.p. 211–213 °C, was synthesized by the procedure of Tanaka[10] and reduced in 91% yield by sodium amalgam in dilute aqueous base[8] to the previously undescribed (±)-5-methoxy dihydro coumarilic acid (**VII**), m.p. 99–102 °C (ex benzene). This acid **VII** was resolved via its amphetamine salts as was done for the unsubstituted acid[8]. The salt obtained with l-amphetamine, m.p. 159–162 °C (ex acetone), yielded the (+)-5-methoxy dihydro coumarilic acid (**VIII**) [m.p. 81–83 °C (ex benzene/pet ether); $[\alpha]_D^{25}$ + 37° ($CHCl_3$)] on treatment with cold 6N HCl. This acid **VIII** was reduced, by 1 h reflux in THF with excess $LiAlH_4$, to the carbinol **IX**

1 Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Ardsley, New York 10502, USA.
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3 B. M. Angrist, G. Sathananthan, H. Thompson and S. Gershon, Curr. Ther. Res. *18*, 359 (1975).
4 D. H. Mielke, D. M. Gallant, G. Bishop and C. M. Kessler, Psychopharmac. Commun. *1*, 117 (1975).
5 T. J. Petcher, J. Schmutz, H. P. Weber and T. G. White, Experientia *31*, 1389 (1975).
6 W. Lippmann, T. Pugsley and J. Merker, Life Sci. *16*, 213 (1975).
7 C. F. Huebner, U. S. Patent 3,759,927 (to Ciba-Geigy).
8 D. M. Bowen, J. I. DeGraw, Jr, V. R. Shah and W. A. Bonner, J. med. Chem. *6*, 315 (1963).
9 W. A. Bonner, N. I. Burke, W. E. Fleck, R. K. Hill, J. A. Joule, B. Sjöberg and L. H. Zalkow, Tetrahedron *20*, 1419 (1964).
10 S. Tanaka, J. Am. chem. Soc. *73*, 872 (1951).